Plant Germplasm Conservation: Perspectives for the 2000s. 101-110 (1994). Taiwan Agr. Res. Institute

THE ROLE OF BIOTECHNOLOGY IN EXPLOITING AND USING PLANT GERMPLASM

Edward Charles Cocking
Plant Genetic Manipulation Group, Department of Life Science,
University of Nottingham, Nottingham NG7 2RD, UK

ABSTRACT

Following the inauguration of this National Plant Genetic Resources Centre, it is particularly timely to assess the role of biotechnology in relation to plant genetic resources conservation now and into the next century. Biotechnology needs access to plant germplasm to enable the desired novel plants and plant products to te produced; conversely plant genetic resources can often greatly benefit from the procedures used in biotechnology. Plant germplasm conservation is central to the strategies, particularly of Developing Countries, with regard to the acquisition and development of plant biotechnology built upon their indigenous capacity fortified by links with the CGIAR system and affiliated centres.

Progressively, the techniques of biotechnology are being applied to the major crops of the world, including wheat, maize, rice, potato, barley, cassava, soybean, sorghum, sugarcane, banana, tomato, cabbage, beans and mango. These techniques range from the relatively simple procedures associated with micropropagation to cell manipulations, coupled with plant regeneration from cultured plant cells and tissues. Cell manipulations centre upon the transfer of genes not only between sexually isolated plant species by protoplast fusion procedures, but also the transfer of genes directly into plant protoplasts, cells and tissues from other plant species, microorganisms or animal cells. The extent to which plant germplasm is being exploited and used in plant biotechnology is well illustrated with reference to the world's most important cereal, rice, and to tomato. Micropropagation is now being increasingly used to conserve wild rice species, and protoplast fusion procedures coupled with the regeneration of rice plants from protoplasts are being used to produce a novel range of improved cytoplasmic and nuclear combinations, including those for cytoplasmic male-sterility, and stress tolerances such as salinity tolerance. Direct gene transfer is being used to transfer genes for

enhanced insect and virus resistance. In tomato, plant regeneration from tissue cultures has been used to improve the sepctrum of germplasm, and protoplast fusion and plant regeneration from cultured cells used to facilitate gene flow from wild tomato species, with desirable features, into cultivated tomatoes. Overall biotechnology should not be viewed as a panacea for problems with existing germplasm but more as an oppotunity for the World to increase its biodiversity by accelerating and enabling novel germplasm production for the benefit of mankind.

INTRODUCTION

At this Symposium on Plant Germplasm Conservation-Perspectives for the 2000s, it is particularly timely, before discussing in detail the role of biotechnology in exploiting and using plant germplasm, to look back over the past decade as we attempt to look forward to the next. At the beginning of the 1980s, although it was clear that plant breeding using well established conventional methods would continue to make the key contribution to meet the demands of increased food production, it was forecast that multiple gene transfer mediated by somatic hybridization would, during the next few decades, assume increasing significance in the improvement of forage legumes, vegetable and fruit crops, It was also suggested that during the next decade it would be advantageous to attempt to incorporate into the recipient crop species some limited, perhaps single, genetic attribute of a donor species using a transformation procedure which would eliminate the need for backcrossing and recurrent selection. It was also forecast that in the more immediate short term practical gains would be made using tissue culture and protoplast cloning to enhance and release somatic variability in plants (Cocking et al., 1981).

Since 1981 most of these forecasts have been realised, but it has also been realised how vital plants and primitive crop varieties or 'land races' are to our ability to utilize these new methods of plant improvement for plant biotechnology. As long as we can protect these resources either by *in situ* conservation or *ex situ* conservation, there is hope that this partnership of modern science and the plant kingdom will flourish and continue to provide all we need for the crowded world of the next century (Huxley, 1984).

Plant cell, tissue and organ cultures

Plant biotechnology has greatly benefitted from the utilization of

methods which do not rely on sexual crossing or cross-hybridization, but which aim to propagate useful cultivars very rapidly and also create new ones with the help of plant cell, protoplast, tissue and organ culture, as well as genetic engineering techniques. Meristem culture and the regeneration of whole plants has been used for the micropropagation of cultivars and virus-free plants, and the culture of protoplasts and haploid tissues, somatic embryogenesis, selection of variants and mutants, protoplast fusion and regeneration of whole plants has been used for micropropagation and the improvement of cultivars. Genetic recombination, gene transfer and regeneration of whole plants have been applied to the improvement of cultivated plants and plant cell culture on a large scale for the production of useful substances (Sasson, 1989). The applications of these procedures to plant germplasm fall into three main categories: short-term applications which require about three years for their implementation, these include vegetative or clonal propagation; medium-term applications, such as the the development of somatic embryogenesis and embryo culture which require up to six or more years for the implementation; and long-term applications which cover gene transfer either through the production of somatic hybrid plants or transgenic plants.

The role of the CGIAR system and affiliated centres

The Consultative Group on International Agricultural Research (CGIAR) established in 1971 is an informal association of some 50 countries, international and regional organizations and private foundations working together to support agricultural research around the world. Today, the CGIAR is a global network of 16 international centres working to increase food production in less developed countries. Plant biotechnologies are increasingly being developed as effective procedures for the exploitation of plant germplasm within the limit of these various international agricultural research centres. At CIAT (the International Centre for Tropical Agriculture) plant cell, tissue and organ culture technology is being applied to improving beans, cassava and rice production for Latin America and the Caribbean, especially acid-tolerant upland rices. The International Maize and Wheat Improvement Centre (CIMMYT) conducts research and training related to the production of maize and wheat, two crops that together account for more than 40% of the cereal grains produced in the developing world. Because many of the procedures associated with the plant cell, tissue and organ culture of both wheat and maize have been more difficult than those for beans,

cassava and rice, progress in the application of biotechnology to the use of maize and wheat germplasm has been slow. At the International Rice Research Institute (IRRI) there is emphasis on the unique opportunities created by plant biotechnology to exploit the procedures of somatic hybridization and transgenic rice producation. The International Potato Centre (CIP) located in Lima, Peru, has taken the lead in collecting, developing and distributing potatoes adapted to the diverse growing conditions of the tropics, where the majority to the developing centres are located. The potato has been shown to be highly responsive to the various procedures for the application of plant cell, tissue and organ culture to potato improvement, utilizing the extensive germplasm of the World Potato Colection maintained at CIP. IRRI has similarly utilized its extensive germlasm collection of rice varieties, and in general, germplasm collections have greatly stimulated plant biotechnology research throughout the world.

The Syria-based International Centre for Agricultural Research in the Dry Areas (ICARDA) has world responsibility for the improvement of barley, lentil and faba bean and a regional mandate for the improvement of wheat, chickpea, pasture and forage crops. Largely because of technical difficulties associated with the development of plant biotechnological procedures for these crops progress has also been slow. A somewhat similar situation applies to the genetic improvement of sorghum, millets, pigeonpea, chickpea and groundnut (peanut) being undertaken at the Internaional Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and of cassava, maize, cowpeas, yams, soybean and plantain at the International Institute of Tropical Agriculture (IITA) outside of Ibadan, Nigeria. The International Network for the Improvement of Banana and Plantain (INIBAP) is particularly well poised to exploit the fact that procedures for the regeneration of banana plants via somatic embryogenesis have been established, (Teisson and De Langhe, 1989).

The exploitation and use of tomato germplasm

The tomato (*Lycopersicon esculentum*) owes its enormous commercial success to its wild relatives. The crop has gained resistance to the fungus *Fusarium oxysporum* from the wild relative *L. pimpinellifolium;* a change to the fruit stalk that adapts it for mechanical harvesting from another wild relative, *L. cheesmanii;* higher beta carotene from the wild relative *L. hirsutumand;* increased vitamin C content from another wild relative *L.*

peruvianum, (Huxley, 1984) The introgression of genes from these wild relatives has been achieved by sexual hybridization, sometimes, as for crosses involving *L. peruvianum*, using embryo rescue. The more extensive utilization of the germplasm of *L. pernvianum* has required the use of protoplast fusion technology.

Species of the genus *Lycopersicon* can be divided into two major groups on the basis of their sexual crossing relationships: a group of species that can be hybridised with the cultivated tomato with relative ease (the *'esculentum* complex'), and another group separated from the cultivated species by severe sexual barriers (the *'peruvianum* complex'). The species of the *'peruvianum* complex' represent a vast store of potentially useful genetic variation which tomato breeders are frequently deterred from utilizing because of the daunting sexual barriers which have to be overcome (Taylor and Al-Kumar, 1982). Gene transfer by protoplast fusion is an attractive additional procedure for transferring genes between members of the *'peruvianum* complex' and the cultivated tomato, and generally for the better exploitation of wild species of *Lycopersicon* because it overcomes the limitations of sexual isolation mechanisms. Protoplasts of *L. esculentum* can be readily isolated directly from tomato plants. The wild species *L. peruvianum* is readily capable of regeneration into plants from protoplast-derived callus (Zapata et al., 1977). Fertile somatic hybrid plants have been produced by the fusion of protoplasts of *L.esculentum* with protoplasts of *L. peruvianum* (Kinsara et al., 1986) and have led to an ability to readily introgress genes from the wild relative *L. peruvianum* into *L. esculentum*. Ongoing opportunity exists for the use of somatic hybridization to introgress other useful genes from other wild relatives of tomato from the extensive range of germplasm available.

Application of strict genetic engineering to a crop species such a tomato depends on the identification, isolation cloning and expression of genes of economic importance, whether these are genes for disease resistance, stress tolerance of improved yield potential. Cultivated tomato (*L. esculentum*) can be readily transformed using *Agrobacterium tumefaciens* as the vector and transgenic plants produced. Single genes can be readily transferred. As discussed by Sasson (1986), the transfer of genes coding for herbicide resistance has attracted financial support from several agrochemical companies, because of the commercial advantages resulting from the innovation. These include expansion of the market for wide spectrum and cheaper herbicides, to include crops previously susceptible, and extension of the period over which a company can continue to profit from the sale of its herbicides, even

after the expiry of patents, by selling them with the seeds of the resistant varieties. Resistance has been transferred to tomato by Holchst A. G.'s phosphinotricin and by Monsanto Co.'s glyphosate, the world's largest-selling herbicide. In addition to the transfer of genes coding for resistance to herbicides, the gene encoding *Bacillus thuringiensis* toxin has been transferred, and pest resistant tomato plants obtained.

In the future an evaluation will be required as to the relative merits of utilizing the natural biodiversity of germplasm available for particular gene transfers in tomato, as compared with the direct transfer of specific genes.

The exploitation and use of rice germplasm

Until recently the sexual input of genes from wild rice species into cultivated rice (*Oryza sativa*) was limited to one particular improvement, but, as recounted by Huxley (1984), with spectacular results. Following the Green Revolution with its associated large-scale paddies cropping twice a year, grassy stunt virus carried by the brown planthopper appeared almost from nowhere as an important disease. Screening the then 6,273 samples of rice kept in the collection of IRRI revealed only one, the feeble *Oryza nivara* that had the required resistance character; as a bonus, it was also resistant to blast, another major disease of rice. This sample of *Oryza nivara* had been collected from central India in 1966, at a time as highlighted by Huxley (1984) when noone would have guessed that it would ever be of value. Now thanks to *Oryza nivara,* IR36 the rice variety with improved virus resistance was once planted across more than 10 million hectares.*

Apart from *Oryza nivara,* wild species within the genus *Oryza* and other relevant Graminaceous species offer sources of important genes conferring traits such as salinity tolerance and resistance to pests and diseases (Chang and Vaughan, 1991). Since, as discussed by Finch et al. (1991), because many of the wild species in question are sexually isolated from cultivated rice, protoplast fusion offers a unique opportunity to develop previously unobtainable hybrids and cybrids, particularly in relation to novel cytoplasmic male-sterility. Furthermore, direct interaction of protoplasts with recombinant plasmids can lead to the incorporation and expression of specific -gene constructs. In this connection, to take advantage of the potential that

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* Note added by the editors: Since the early 1980s, the *Gsv* gene from *O. nivara* has been rendered ineffective by the appearance of biotype 2 of the grassy stunt virus.

array of characters that breeders are concerned about in population or variety improvement programmes must be mapped. To provide breeders with a useful repertoire of new tools, a saturated linkage map based on both molecular markers and phenotypic characters of agronomic importance must be available. As discuseed by McCouch and Tanksley (1991), as the current rice map evolves towards saturation, and new technologies give rise to new types of genetic markers and new possibilities for locating and locating genes of interest, the opportunities for effectively integrating molecular analysis of genetic variation into existing plant improvement programmes become increasingly apparent.

Protoplast fusion and transformation leading to the production of transgenic plants are techniques which have previously been limited mainly to the dicotyledons. However, recent successes in plant regeneration from protoplasts (Lynch et al., 1991) have enabled the coupling of protoplast to plant technology with recombinant DNA and fusion technologies, thus linking rice breeding and biotechnology. Finch et al. (1990) obtained dividing heterokaryons from fused protoplasts of the japonica rice variety Taipei 309 and mesophyll protoplasts of the important wild rice relative, *Porteresia coarctata*. The latter species is highly salt tolerant, but is sexually isolated from tribe Oryzeae; this work forms a basis for the somatic hybridization of these two important species providing the opportunity for gene flow for salt tolerance into cultivated rice. The first successes in the production of transgenic rice plants from protoplasts by direct interaction of plasmids with protoplasts were reported in 1988 for japonica rice varieties (Hodges et al., 1991). The transfer of a specific gene or group of genes into cultivated rice has a wide potential application in the genetic manipulation of rice. Potentially useful genes for rice genetic engineering include those for enhanced yield potential, pest resistance, abiotic stress tolerance, improved product quality, and lowered production costs (Toenniessen, 1991).

As for tomato and the other major crops of the world including wheat, maize, potato, barley, cassava, soybean, sorghum, sugarcane, banana, cabbage and beans, in the future an evaluation will be required for rice as to the relative merits of utilizing the natural biodiversity of germplasm available for particular gene transfers as compared to the direct transfer of specific genes obtained from other sources.

Cryopreservation and *in vitro* propagation

The seeds of most of the major crops are orthodox (nonrecalcitrant) and

can be refrigerated, and therefore in this regard cryopreservation in liquid nitrogen (-196°C) is of limited value. For some recalcitrant seeds, particularly those of semiarid lands, such cryopreservation is particularly useful. Cell cultures used in various plant genetic manipulations on repeated subculturing undergo genetic erosions, polyploidy, gene amplification and chromosome elimination changes; cryopreservation at -196 °C holds great promise to maintain their genetic stability. Such cryopreservation has, for instance, been very effectively used to maintain the plant regeneration capability of rice cell suspension cultures, and of rice protoplasts isolated from cell suspension cultures. This facility has helped greatly in enabing laboratories in developing countries to benefit from this technology transfer in the Rice Biotechnology Program of the Rockefeller Foundation (Lynch et al., 1991).

Clonal propagation of shoots *in vitro,* or micropropagation, allows the rapid multiplication of a particular plant species in a sterile, disease-free environment whilst minimising somaclonal variation, and is thus a particularly useful technique for the multipication or conservation of male-sterile or rare genotypes. An example is the recent development of a method for the *in vitro* clonal propagation of shoots from a range of wild rices, and other grass species, that have important genetic traits such as drought resistance and salinity tolerance (Finch et al., 1992). The axenic multiple shoot cultures could be maintained without subculture for several months, or rapidly multiplied, for the subsequent production of mature plants and seeds. It is envisaged that this clonal propagation technique could aid the genetic manipulation of cultivated rice by providing a means to vegetatively conserve valuable genetic resources, a technique to rapidly multiply novel hybrid material, and a source of embryogenic callus that will allow the application of biotechnological techniques, such as somatic hybridization and genetic transformation to perviously unexploited species.

ACKNOWLEDGEMENTS

The financial support of the UK Overseas Development Administration and the Rockefeller Foundation is gratefully acknowledged for work on Rice Biotechnology at the University of Nottingham (Plant Genetic Manipulation Group).

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